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Comparative Activities of New Fluoroquinolones, Alone or in Combination with Amoxicillin, Trimethoprim-Sulfamethoxazole, or Rifampin, against Intracellular *Listeria monocytogenes*

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We studied the activities of the new fluoroquinolones clinafloxacin, levofloxacin, ofloxacin, and sparfloxacin alone or in combination on the intracellular growth of *Listeria monocytogenes*. Against intracellular growth of the four strains tested, a similar reduction of the bacterial count was obtained with clinafloxacin at the dose of 10× MIC ($\Delta\log_{10}$ CFU/ml = −2.19 ± 0.24), with levofloxacin at 8× MIC ($\Delta\log_{10}$ CFU/ml = −2.28 ± 0.25), and with sparfloxacin at 4× MIC ($\Delta\log_{10}$ CFU/ml = −2.16 ± 0.21) after 24 h of incubation. The combination of the quinolones with trimethoprim-sulfamethoxazole or amoxicillin did not show a substantial increase in activity compared to the fluoroquinolone alone. Antagonism with rifampin was strongly suggested. No modification of the MIC was observed after 20 successive infections of HeLa cells and contact with subinhibitory concentrations of clinafloxacin, levofloxacin, and sparfloxacin for 24 h. We conclude that clinafloxacin, levofloxacin, or sparfloxacin could represent a therapeutic alternative to amoxicillin for the treatment of *Listeria* infections in adults, especially clinafloxacin, whose MIC is low (0.06 to 0.12 μg/ml), and whose best activity against intracellular *L. monocytogenes* was obtained at a concentration of 1.2 μg/ml, which is similar to clinically achievable levels. The results must be confirmed in an experimental model.

Listeria monocytogenes is a Gram-positive bacillus which grows in the cytosol of macrophages (10). It can also multiply in vitro in nonphagocytic cells, such as fibroblasts or epithelial cells (5). *L. monocytogenes* infections, although uncommon except in the context of outbreaks due to contaminated food in adults, raise therapeutic problems in view of the high mortality, especially in the case of meningoencephalitis and the absence of any therapeutic alternative clearly demonstrated to be effective in the case of penicillin allergy. The mortality is often related to an underlying disease. During the 1992 French foodborne epidemic of listeriosis, related to the consumption of jellied pig tongue, the mortality rate was 40.8% (51 of 125) in the presence of underlying disease and 12.9% (8 of 62) in the absence of any underlying disease in patients over the age of 65 (no unfavorable outcome was observed in patients younger than 65 years) (11). Although the standard treatment for *Listeria* infections remains the ampicillin-gentamicin combination, many authors have evaluated other therapeutic alternatives in open, nonrandomized, and noncomparative studies. Trimethoprim-sulfamethoxazole (TMP-SMX), which is known to have good intrathecal penetration (9), used alone in the case of penicillin allergy or in combination with amoxicillin, appears to give results comparable to those obtained with the amoxicillin-gentamicin combination (15, 18). In a previous report, we developed a in vitro model to study the activities of antibiotics on intracellular *L. monocytogenes* in HeLa cell cultures (12). In this study, amoxicillin, sparfloxacin at 5 μg/ml, and the trimethoprim-sulfamethoxazole plus gentamicin and sparfloxacin plus gentamicin combinations were found to be the most active treatments, but the bacterial reduction did not exceed 2 $\log_{10}$ and these antibiotics did not allow complete eradication of bacteria in this nonphagocytic cell model. New fluoroquinolones have excellent intracellular penetration and are essentially concentrated in the cytosol of macrophages with a rapid and intense efflux (5, 19). Some fluoroquinolones, whose spectrum includes *Listeria* species and other gram-positive bacteria, could represent a therapeutic alternative provided their meningeal diffusion is sufficient. We therefore studied, in vitro, the efficacies of four fluoroquinolones at different doses on various strains of *L. monocytogenes*, under extracellular conditions and after intracellular infection of HeLa cells.

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MATERIALS AND METHODS

Bacterial strains and culture media. The reference strain of *L. monocytogenes* used first was the hemolysin-producing strain EGD, serotype 1/2a, derived from the Institut Trudeau (1982) (10). It was grown in tryptic soy broth and stored at −80°C in the form of 1-ml aliquots containing 4.8×10^8 bacteria per ml in the growth phase.

Ten wild strains of *L. monocytogenes*, isolated in 1991 and 1992 from blood or cerebrospinal fluid (CSF) of patients admitted to our institution (Hôpital Pontchaillou, Rennes, France), were also used for the determination of MICs and minimal bactericidal concentrations (MBCs) and 3 of them, considered representative of these clinical isolates, were used as controls for the results observed with EGD. Their identification and serotype were verified by the Centre National des Listeria (Nantes, France), where they are identified in the collection by the following numbers: CNL 920492 (serotype 1/2a), CNL 920235 (serotype 4b), CNL 920427 (serotype 1/2a), CNL 920205 (serotype 4b), CNL 920226 (serotype 4b), CNL 920467 (serotype 4b), CNL 920290 (serotype 1/2b), CNL 910351 (serotype 1/2a), and CNL 92005 (serotype 1/2a). For each experiment, the aliquots necessary for bacterial inoculation were rapidly thawed and adjusted in minimum essential medium culture medium (MEM) containing Earle salts (Flow Laboratories, Irvine, Scotland), supplemented with 1% essential amino acids, (Flow Laboratories) and 2% glutamate (Laboratoire Eurobio, Les Ulis, France), to which bicarbonate was added in order to obtain a constant pH of the solution equal to 7.2. *Staphylococcus aureus* ATCC 25922 was used as the reference strain in in vitro antibiotic susceptibility testing.

Antibiotics. The following antibiotics were provided by the manufacturers in the form of powders suitable for susceptibility testing: amoxicillin (SmithKline

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Beecham), sparfloxacin (Rhône Poulenc), levofloxacin and ofloxacin (Roussel Uclaf), clinafloxacin (Parke-Davis), rifampin (Merrel), and TMP-SMX at a ratio of 1:5 (co-trimoxazole; Sigma). The concentrations required to study the activity of the antibiotic on intracellular *L. monocytogenes* and in the culture broth were adjusted on the day of the study using modified MEM supplemented with 10% fetal calf serum (FCS) (TechGen, Les Ulis, France). The activity of the antibiotics was measured at concentrations corresponding to multiples of the MIC.

MIC and MBC. MICs were determined by the broth dilution technique on tryptic soy microplates (Falcon, Becton-Dickinson, Labware, Lincoln Park, Ill.), with Mueller-Hinton broth (Diagnostics Pasteur, Marnes La Coquette, France) according to the procedures published by the National Committee for Clinical Laboratory Standards (14). An inoculum of 10^6 bacteria in log phase of growth per spot was used. The MBC, determined after inoculation of 0.01 ml of each well with no visible growth onto Mueller-Hinton agar by means of Steers' apparatus, was defined as the lowest concentration of antibiotic which decreased the number of viable bacteria by 3 $\log_{10}$.

Bactericidal growth curves. The bactericidal kinetics over 24 h were determined with a mean inoculum of 10^6 of *L. monocytogenes* per ml in tryptic soy broth. After incubation at 37°C with each concentration of the test antibiotics, the number of bacteria was counted after 2, 6, and 24 h of contact by spreading 0.1 ml of the solution obtained after 10-fold dilution in distilled water onto tryptic soy agar (Diagnostics Pasteur). The limit of sensitivity was defined as at least 1 dilution (>100 CFU/ml) to avoid any carryover effects. The activities of the antibiotics tested were compared in terms of their $T_{\log}$, the time necessary to reduce the bacterial population by 1 log unit, and the minimal bactericidal time (MBT), the time necessary to reduce the bacterial population to 4 $\log_{10}$ survivors (7).

Maintenance and infection of HeLa cells. The HeLa cell line was maintained as previously described (12). The number of cells infected by *L. monocytogenes* was counted at each experiment and ranged between 8×10^5 and 2.5×10^6 per well. The semiconfluent monolayer of HeLa cells was inoculated with a suspension of *L. monocytogenes* adjusted in MEM (without addition of FCS) in order to obtain an infection density of 50 bacteria per cell. After incubation for 1 h at 37°C, the infected cells were washed five times with phosphate-buffered saline (PBS) and covered with 2 ml of MEM supplemented with 10% FCS and 5 mg of gentamicin per liter to kill the extracellular bacteria. After 90 min of contact, the cells were again washed five times with PBS and covered with 2 ml of the test concentration of antibiotic or antibiotic combination and were incubated for another 24 h. After being washed five times in PBS to remove any extracellular antibiotic, the infected cells were lysed in 2 ml of distilled water maintained on crushed ice for 15 min. The viable intracellular bacteria were counted by spreading 0.1 ml of the solution obtained after 10-fold dilution in distilled water onto tryptic soy agar, after 90 min of contact with gentamicin (T_0), and after 2 h (T_2), 6 h (T_6), and 24 h (T_{24}) of contact with the test antibiotic. A control test without antibiotic was performed at each manipulation. The activity of each concentration of antibiotic was usually determined simultaneously for two different strains and always in three culture wells per strain (in triplicate) in each experiment. The results for each strain in one experiment were expressed as the mean in $\log_{10}$ of the number of viable bacteria in each of the three wells. The results reported here were obtained after several separate experiments performed at different times. They were expressed as the geometric means in $\log_{10} \pm$ standard deviation for the four tested strains. The efficacy of clinafloxacin at the concentrations of 0.6 and 1.2 μg/ml was tested in seven separate experiments (two strains used in each experiment), and five separate experiments tested the efficacy of levofloxacin (at the concentration of 1 to 4 μg/ml), sparfloxacin (at 2 to 8 μg/ml), the amoxicillin plus clinafloxacin, and TMP-SFX plus clinafloxacin combinations. The other presented results were checked by at least three different experiments under the same conditions for EGD and were performed at least once for each of the three clinical strains.

Selection of resistance by serial passages with subinhibitory concentrations. We also studied the MIC for two clinical strains of *L. monocytogenes* after 20 successive infections of HeLa cells incubated for 24 h in the presence of subinhibitory concentrations of clinafloxacin (0.3 μg/ml), levofloxacin (1 μg/ml), and sparfloxacin (2 μg/ml). After each experiment, a colony of *L. monocytogenes* was collected on tryptic soy agar and allowed to grow in broth for 18 h on the day before the following manipulation. After adjustment in MEM to obtain an inoculum between 4×10^7 and 8×10^7/ml, new HeLa cells were infected by the same technique as that described above.

Statistical analysis. All results are expressed as the mean ± standard deviation, including all of the results obtained in each separate experiment with the four tested strains. Comparisons between groups were performed by two-way analysis of variance with one independent factor, the treatment, and one paired factor (MIC). The Student *t* test was used for comparing the efficacy of each antibiotic alone or in combination two by two.

RESULTS

In vitro susceptibilities of extracellular *L. monocytogenes* to fluoroquinolones. The MICs and MBCs of the four quinolones tested were determined for the 10 wild strains and strain EGD. The MICs of clinafloxacin ranged from 0.06 to 0.12 μg/ml and the MBCs ranged from 0.12 to 0.25 μg/ml. The MBC/MIC ratio was always less than or equal to 2. For levofloxacin, the MICs were higher, between 0.25 and 0.5 μg/ml, the MBC was 0.5 μg/ml, and the MBC/MIC ratio was usually equal to 1. For ofloxacin, the MICs ranged from 0.25 to 2 μg/ml, the MBC was equal to 2 μg/ml, and the MIC/MBC ratio was equal to 2. For sparfloxacin, the MIC was 2 μg/ml for all strains, the MBC was equal to 4 μg/ml, and the MBC/MIC ratio was usually equal to 2. The in vitro bactericidal effect in tryptic soy broth was determined for the EGD strain, the epidemic strains CNL 920205 and CNL 920467 derived from CSF, and the sporadic strain CNL 920290 derived from a blood culture. The bactericidal activity curves, presented in Fig. 1, correspond to the mean values for the bactericidal activity tests performed with EGD. The results are expressed as the mean values for the four strains of *L. monocytogenes* with a comparable initial inoculum [$(4 \pm 3) \times 10^6$/ml]. Rapid bacterial reduction was obtained with clinafloxacin at concentrations of 0.12 to 2 μg/ml. The $T_{\log}$ was less than or equal to 2 h for concentrations greater than or equal to 2× MIC ($\Delta \log_{10}$ CFU/ml = -1.12 ± 0.64, with a concentration of 0.3 μg/ml). The MBT was more than 6 h for concentrations of 0.6 μg/ml (5× MIC) and 1.2 μg/ml (10× MIC) with strain EGD (Fig. 1). After 24 h of incubation, the decrease in bacterial population was -3.41 ± 0.46 $\log_{10}$ CFU/ml with a concentration of 2× MIC, -3.79 ± 0.52 $\log_{10}$ with a concentration of 5× MIC, and -3.9 ± 0.10 $\log_{10}$ with 10× MIC. At the concentration of 0.06 μg/ml, the number of bacteria surviving after 24 h of incubation was close to that of the control.

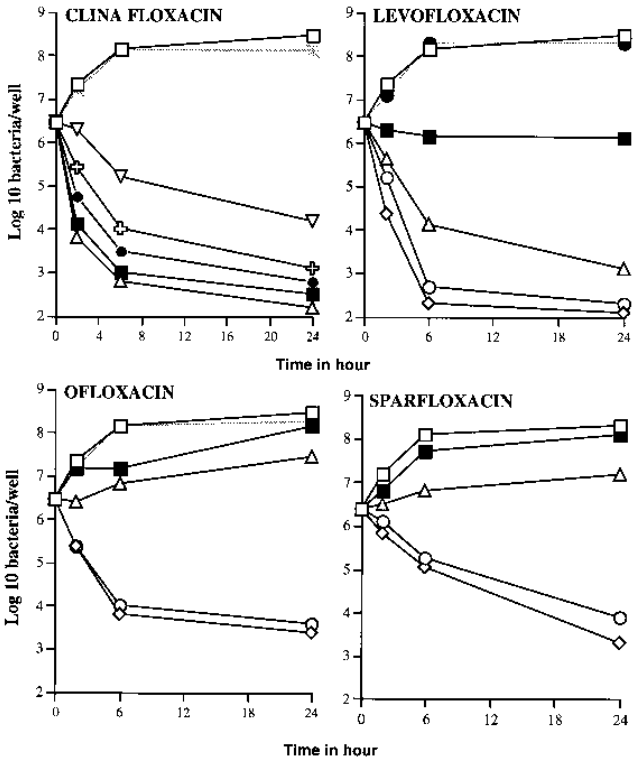

FIG. 1. Activities of clinafloxacin, levofloxacin, ofloxacin, and sparfloxacin on *L. monocytogenes* EGD cultured in tryptic soy broth. The bacterial counts were determined at 0, 2, 6, and 24 h of incubation with different concentrations. Symbols: □, control; ◇, 8 μg/ml; ○, 4 μg/ml; △, 2 μg/ml; ■, 1.2 or 1 μg/ml; ●, 0.5 or 0.6 μg/ml; ✣, 0.25 μg/ml; ▽, 0.12 μg/ml; ✳, 0.06 μg/ml.

Levofloxacin decreased the *Listeria* population by more than

3 $\log_{10}$ with concentrations greater than or equal to 2 μg/ml after 24 h of incubation. The $T_{\log}$ of levofloxacin was 2 h ($\Delta\log_{10}$ CFU/ml = -1.2 ± 0.25) for concentrations greater than or equal to 4 μg/ml (8× MIC), but MBT was more than 24 h ($\Delta\log_{10}$ CFU/ml = -3.51 ± 0.5 at a dose of 4 μg/ml). After 24 h of incubation with a concentration of 2 μg/ml (4× MIC), levofloxacin reduced the number of bacteria surviving to -3 ± 0.57 $\log_{10}$ CFU/ml. At the concentration of 1 μg/ml, levofloxacin was only bacteriostatic, and at the concentration of 0.5 μg/ml, the activity curve was close to that observed with the control. Ofloxacin was only active at the concentration of 4 μg/ml ($\Delta\log_{10}$ CFU/ml = -2.53 ± 0.3 after 24 h of incubation). Sparfloxacin was bacteriostatic at a concentration of 2 μg/ml. With a concentration of 2× MIC (4 μg/ml) and 4× MIC (8 μg/ml), the number of bacteria surviving after 24 h of contact was -2.02 ± 0.81 and -2.77 ± 0.2 $\log_{10}$, respectively.

We also studied the MIC for two clinical strains, CNL 920205 and CNL 920467, after 20 successive infections of HeLa cells incubated for 24 h in the presence of subinhibitory concentrations of clinafloxacin, levofloxacin, and sparfloxacin. Following these 20 passages in the presence of one of the three quinolones, the MIC remained the same as the initial MIC.

In vitro susceptibilities of the 10 strains of *L. monocytogenes* to the other antibiotics used in this study. The MIC of amoxicillin was 0.03 μg/ml (but the MBC was equal to 2 μg/ml, and the results were inoculum dependent). The MICs of rifampin and TMP-SMX (ratio, 1:5) for all tested strains were 0.12 and 0.25 μg/ml, respectively.

Intracellular activities of quinolones against *L. monocytogenes*. Study of the viability of HeLa cells infected by *L. monocytogenes* has been previously conducted (12). Before each manipulation, the integrity of the cell layer covering the well was verified, and the bacterial count was not performed when significant detachment of the cell layer was observed and when the efficacy of tested antibiotics had been previously proven. Strains EGD, CNL 920205 (serotype 4b), and CNL 920467 (serotype 4b) derived from CSF and the sporadic strain CNL 920290 (serotype 1/2b), a blood isolate, were studied. The activities of the four fluoroquinolones tested, presented in Fig. 2, correspond to the mean results obtained with the four strains. The activity obtained with clinafloxacin was dose dependent with an activity threshold: identical results were obtained with concentrations of 1.2 and 2 μg/ml. The bacterial reduction was rapid. ΔLog_{10} CFU/ml was -1.09 ± 0.15 by the second hour at a concentration of 1.2 μg/ml (10× MIC) and -0.7 $\log_{10}$ at a concentration of 0.6 μg/ml (Fig. 2). At the 24th hour, the $\Delta\log_{10}$ CFU/ml was greater than -2 for concentrations equal to or greater than 0.9 μg/ml (data not shown on Fig. 2 for this concentration). The bacterial decrease in the presence of clinafloxacin was -1.42 ± 0.38, -2.05 ± 0.25, and -2.19 ± 0.24 $\log_{10}$ CFU/ml at concentrations of 0.6, 0.9, and 1.2 μg/ml, respectively. Clinafloxacin was only bacteriostatic at a concentration of 0.25 μg/ml.

Levofloxacin was active at the highest concentrations: the bacterial reduction was greater than 2 $\log_{10}$ at the concentration of 4 μg/ml, and the bacterial reduction after 24 h of incubation was -2.28 ± 0.25 and -1.38 ± 0.10 $\log_{10}$ CFU/ml at concentrations of 4 and 2 μg/ml, respectively. Levofloxacin was inactive at the concentration of 2× MIC ($\Delta\log_{10}$ CFU/ml = 1.32 ± 0.22 at 1 μg/ml). Ofloxacin induced only a modest bacterial reduction at 24 h: reduction of surviving bacteria at the concentration of 4 μg/ml was -0.40 $\log_{10}$ CFU/ml.

Similar results were obtained for sparfloxacin, whose peak activity was rapidly obtained at the 6th hour at the dose of 4 μg/ml ($\Delta\log_{10}$ CFU/ml = -1.03 ± 0.23) and at the dose of 8 μg/ml ($\Delta\log_{10}$ CFU/ml = -2.16 ± 0.21).

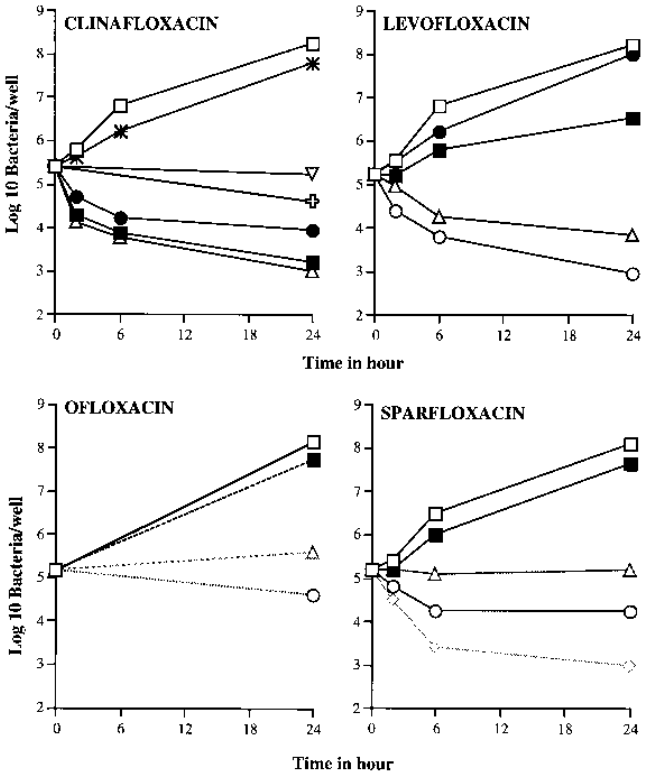

FIG. 2. Activities of clinafloxacin, levofloxacin, ofloxacin, and sparfloxacin on intracellular *L. monocytogenes*. The bacterial counts were determined at 0, 2, 6, and 24 h of incubation with different concentrations. Symbols: □, control; ◇, 8 μg/ml; ○, 4 μg/ml; △, 2 μg/ml; ■, 1.2 or 1 μg/ml; ●, 0.6 μg/ml; ✣, 0.25 μg/ml; ▽, 0.12 μg/ml; ✳, 0.06 μg/ml.

Intracellular activities of antibiotic combinations on *L. monocytogenes*. The activities on intracellular *L. monocytogenes* of amoxicillin alone at the concentration of 2.4 μg/ml, rifampin at the concentration of 10 μg/ml, and TMP-SMX at the concentration of 100 μg/ml were identical to those described previously (12). The reduction of bacterial count after 24 h of incubation was 1.8 $\log_{10}$ CFU/ml ± 0.26 with amoxicillin, 0.90 $\log_{10}$ CFU/ml ± 0.24 with rifampin, and 1.70 $\log_{10}$ CFU/ml ± 0.21 with TMP-SMX.

For the combination we tested the doses at 5× and 10× MIC for clinafloxacin, 2× and 8× MIC for levofloxacin, and 1× and 4× MIC for sparfloxacin.

Some combinations were found to be slightly more bactericidal than fluoroquinolone used alone, but there was no statistical difference ($P > 0.05$) except for some combinations with rifampin that were antagonistic (Fig. 3 and 4). After 24 h of incubation, the reduction of the bacterial count was -1.73 ± 0.30 and 2.37 ± 0.19 $\log_{10}$ CFU/ml with the combination of clinafloxacin (0.6 μg/ml) plus amoxicillin (2.4 μg/ml) and clinafloxacin (1.2 μg/ml) plus amoxicillin (2.4 μg/ml), respectively. The results of the combination of rifampin (10 μg/ml) plus clinafloxacin (0.6 μg/ml) showed a trend of antagonism, as the bacterial reduction was identical to that obtained with rifampin alone ($\Delta\log_{10}$ CFU/ml = -1.09 ± 0.11) and lower than that obtained with clinafloxacin alone ($\Delta\log_{10}$ CFU/ml = -1.42 ± 0.36 in the same experiment) but no statistical difference was found ($P = 0.14$). When combined with a higher concentration of clinafloxacin (1.2 μg/ml), rifampin induced a $\Delta\log_{10}$ CFU/ml of -1.64 ± 0.66 lower than that induced by clinafloxacin alone ($P = 0.059$) (Table 1). No substantial ben-

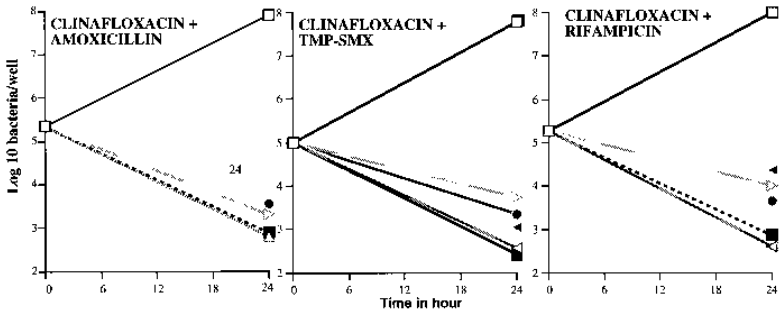

FIG. 3. Bacterial activities of amoxicillin, TMP-SMX, and rifampin in combination with clinafloxacin against intracellular *L. monocytogenes*. The bacterial counts were determined at 0 and 24 h of incubation. Symbols: □, control; ■, clinafloxacin at 1.2 μg/ml; ●, clinafloxacin at 0.6 μg/ml; ▷, amoxicillin at 2.4 or TMP-SMX at 100 or rifampin at 10 μg/ml; △, clinafloxacin at 1.2 plus amoxicillin at 2.4 μg/ml; ●, clinafloxacin at 0.6 plus amoxicillin at 2.4 μg/ml; ◁, clinafloxacin at 1.2 plus TMP-SMX at 100 or plus rifampin at 10 μg/ml; ◀, clinafloxacin at 0.6 plus TMP-SMX at 100 or plus rifampin at 10 μg/ml.

efit resulted from the combination of clinafloxacin with TMP-SMX compared to clinafloxacin alone at the same dose (Fig. 3). Levofloxacin, at the concentration of 4 mg/liter, induced a $\Delta\log_{10}$ CFU/ml of -2.24 ± 0.39 in combination with amoxicillin (2.4 μg/ml) and a $\Delta\log_{10}$ CFU/ml of -2.39 ± 0.14 in combination with TMP-SMX (100 μg/ml). The rifampin-levofloxacin combination was antagonistic in this model ($\Delta\log_{10}$ CFU/ml $= -1.3 \pm 0.19$) ($P < 0.0001$). At concentrations of 2, 4, or 8 μg/ml, sparfloxacin in combination with amoxicillin induced a $\Delta\log_{10}$ CFU/ml of -1.44 ± 0.43 to -2.33 ± 0.43, identical to or even lower than that obtained with amoxicillin alone in the same experiment for the dose at 1× and 2× MIC and much higher with the dose at 4× MIC. The bacterial decrease after 24 h of incubation was identical with the combination sparfloxacin plus TMP-SMX and sparfloxacin alone at dose of 8 μg/ml (Fig. 4). In combination with rifampin, sparfloxacin induced a similar reduction of the bacterial count at the dose of 2 and 8 μg/ml (Table 1 and Fig. 4), suggesting a reduction in the activity of sparfloxacin at the higher dose when it was added to rifampin ($P = 0.02$).

DISCUSSION

This study demonstrates the excellent activity of clinafloxacin against virulent strains of *L. monocytogenes*, with low MICs, between 0.06 and 0.12 μg/ml, for all 10 clinical strains tested and for the reference strain EGD. Clinafloxacin was rapidly bactericidal in tryptic soy broth: the $T_{\log}$ was equal to 2 h at 2× MIC. But the decrease of the bacterial count did not reach 4 $\log_{10}$ after 24 h of incubation with the highest concentrations (10× MIC). The intracellular penetration of clinafloxacin was excellent, and its intracellular anti-*Listeria* activity was also rapid (reduction of the bacterial count equal to -1 $\log_{10}$ after 2 h of incubation with 1.2 μg/ml) and intense, although not allowing total bacterial eradication and dose dependent up to 1.2 μg/ml ($P < 0.001$). The activity of clinafloxacin was superior to that of amoxicillin, which demonstrated as lower bactericidal activity and a more limited activity against intracellular *L. monocytogenes* ($\Delta\log_{10}$ CFU/ml $= -1.81$ after 24 h of incubation). No statistically significant reduction of the bacterial count was found by the combination of clinafloxacin with amoxicillin or TMP-SMX ($P = 0.41$ over clinafloxacin alone). The excellent intraphagocytic activity of clinafloxacin has already been demonstrated by Anderson and Joone (1). Peak concentrations in serum obtained with healthy adults after ingestion of 100 and 200 mg of clinafloxacin were 0.89 and 2.5 μg/ml, respectively (i.e., concentrations equal to or greater than those shown to be effective on *L. monocytogenes* in our model using HeLa cell cultures) (6). In the experimental model of pneumococcal meningitis resistant to penicillin and ceftriaxone, clinafloxacin was found to be the most effective antibiotic in monotherapy with CSF sterilization of all five rabbits studied, reflecting good meningeal diffusion in animals (8). The blood/CSF clinafloxacin concentration ratio in this model was 4.9 at the peak and 1 at the trough.

Levofloxacin was only active at the highest concentrations against intracellular *Listeria* species. For achieving the same activity as clinafloxacin at a dose of 1.2 μg/ml (10× MIC),

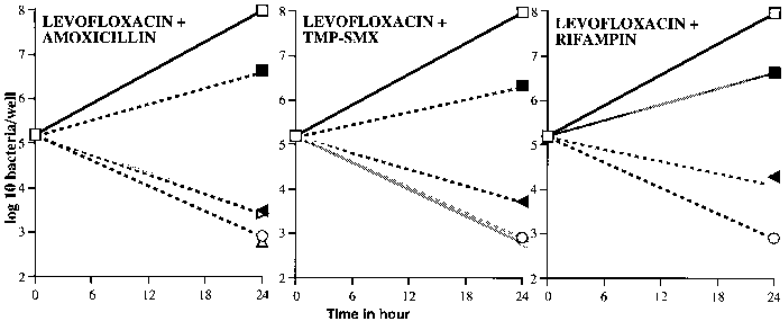

FIG. 4. Bacterial activities of amoxicillin, TMP-SMX, and rifampin in combination with levofloxacin against intracellular *L. monocytogenes*. The bacterial counts were determined at 0 and 24 h of incubation. Symbols: □, control; ○, levofloxacin at 4 μg/ml; ■, levofloxacin at 1 μg/ml; ▷, TMP-SMX at 100 μg/ml or amoxicillin at 2.4 or rifampin at 10 μg/ml; ▲, TMP-SMX at 20 plus levofloxacin at 1 μg/ml; ◩, TMP-SMX at 20 plus levofloxacin at 4 μg/ml; ◁, levofloxacin at 4 plus TMP-SMX at 100 or rifampin at 10 μg/ml; △, levofloxacin at 4 plus amoxicillin at 2.4 μg/ml; ●, levofloxacin at 4 plus amoxicillin at 2.4 μg/ml; ◀, levofloxacin at 1 plus TMP-SMX at 100 or plus rifampin at 10 μg/ml.

TABLE 1. Activities of clinafloxacin, levofloxacin, and sparfloxacin alone and in combination with amoxicillin, rifampin, or TMP-SMX against intracellular *L. monocytogenes* after 24 h of incubation

Antibiotic (μg/ml)	Activity[a]			
	Quinolone alone	Amoxicillin (2.4 μg/ml)	Rifampin (10 μg/ml)	TMP-SMX (100 μg/ml)
Clinafloxacin				
0.6	−1.42 ± 0.38	−1.73 ± 0.30	−1.08 ± 0.11	−1.78 ± 0.37
1.2	−2.19 ± 0.24	−2.37 ± 0.19	−1.64 ± 0.66	−2.22 ± 0.1
Levofloxacin				
1	1.32 ± 0.22	−1.53 ± 0.30	−0.92 ± 0.19	−1.48 ± 0.54
4	−2.28 ± 0.25	−2.24 ± 0.39	−1.3 ± 0.43	−2.3 ± 0.46
Sparfloxacin				
2	0.2 ± 0.13	−1.44 ± 0.43	−1.62 ± 0.15	−1.73 ± 0.10
8	−2.16 ± 0.21	−2.33 ± 0.43	−1.50 ± 0.19	−2.39 ± 0.15

[a] Activities were expressed as the mean in $\log_{10}$ decrease and variance between the four tested strains (standard deviation).

levofloxacin must be used at a dose of 4 μg/ml (8× MIC), which suggests that the intracellular activity was essentially due to the value of the MIC and that the intracellular penetration of these two quinolones was similar. The activity of the levofloxacin (4 μg/ml) plus TMP-SMX (100 mg) combination was identical to that of levofloxacin alone, but rifampin and levofloxacin were antagonistic. However, the mean levels of levofloxacin in human serum usually obtained are 1.22 to 1.36 μg/ml after a dose of 100 mg of levofloxacin and 2.04 μg/ml after a dose of 200 mg (13). A recent population pharmacokinetic study with patients with serious community-acquired infections showed that the mean peak concentration after a dosing schedule of 500 mg every 24 h was 8.67 ± 3.99 μg/ml, which was more than the concentration found to be active on *L. monocytogenes* in this model of HeLa cell cultures (16). Ofloxacin exerted a limited activity against *L. monocytogenes* at usual concentrations. Sparfloxacin demonstrated a moderate activity at concentrations equal to 2× MIC, but at 4× MIC, the reduction of the bacterial count of intracellular *L. monocytogenes* was identical to that obtained with clinafloxacin at a dose equal to 10× the MIC. These data suggested the excellent penetration of sparfloxacin in the cytosol of the HeLa cells, as has been demonstrated in macrophages. Nevertheless, the peak level in serum achieved after oral doses of sparfloxacin in humans is less than what has been shown in the present study to be needed for intracellular activity (17). Previous studies showed that ciprofloxacin was not active at the usual concentrations; this was confirmed in the animal model. An absence of eradication of *Listeria* infection in the spleen and liver in mice pretreated with hydrocortisone, in contrast with ampicillin, was shown (20). No animals survived after ciprofloxacin treatment in the experimental model of *Listeria* meningitis in the gerbil, while 80% of animals survived when treated with amoxicillin and 100% survived when treated with TMP-SMX (2). However, none of the fluoroquinolones, alone or in combination, ensures complete eradication of intracellular *L. monocytogenes.*

The selection of mutant strains of intracellular *L. monocytogenes* by fluoroquinolones did not appear with a mean inoculum of 5.10^7/ml: we demonstrated the absence of elevation of the MIC of levofloxacin, clinafloxacin, and sparfloxacin after 20 successive passages on HeLa cells in the presence of subinhibitory concentrations of these three antibiotics. However, the appearance of strains of *L. monocytogenes* resistant to tetracyclines and a strain resistant to TMP-SMX has been recently reported, and the possibility of a transfer of the gastrointestinal *Enterococcus* gene has been proposed (4). The activity of clinafloxacin alone and in combination with amoxicillin and TMP-SMX should therefore be tested in the experimental model of meningitis in animals. The activity of TMP-SMX may be underestimated in vitro in MEM, as illustrated by the difference of activity obtained with animals (2, 12). This model of investigation of the activity of antibiotics on intracellular *L. monocytogenes* gives very reproducible results. These results are also closely correlated with the experimental model (2).

In conclusion, we have demonstrated that clinafloxacin, alone or in combination with amoxicillin or TMP-SMX, could represent a therapeutic alternative. Further studies with animals and studies of the CSF penetration of clinafloxacin in humans are necessary.

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